latory insufficiency—for example, after myocardial infarction or in cases of arterial insufficiency of a leg.

Spot C was detected in about 60% of the urine samples from carriers of Duchenne muscular dystrophy. Only rarely (4% of cases) were traces of it seen in the electropherograms of urine from normal subjects (table). In these cases the presence of the protein in the urine may possibly have been due to mild muscle damage from exercise, sprains, or falls, etc.

The protein responsible for spot C does not appear to be identical with any of the muscle enzymes known to be present in the serum of patients with muscular dystrophy. In this respect it is interesting that no spot corresponding to creatine kinase could be detected on electropherograms of the urine of these patients, even though spot C was clearly identifiable. On the other hand, unlike spot C, creatine kinase was clearly visible as a protein-staining spot on electropherograms of serum from these patients.

Our studies thus indicate that the presence in the urine of protein migrating as spot C is characteristic of diseases in which the muscle is clearly affected. The detection of this protein in urine, particularly when methods for its radioimmune assay have been developed, might be a useful sensitive aid in confirming the diagnosis of muscle disease and under certain conditions in assessing muscle damage. It might also have a role in the detection of the carrier state in Duchenne muscular dystrophy. We are now attempting to isolate the protein from the urine of boys with Duchenne muscular dystrophy in sufficient amounts to permit its identification.

Prevalence of spot C in electropherograms of urine from normal subjects and patients with neuromuscular diseases

	Age (years)		Total No studied	No with spot C
	Range	Mean		
Normal subjects				
Adult women	18-55	27·5	34	2
Pregnant women	18-33		24	0
Adult men	20-35	28·2	25	0
Boys	6-18	10·0	26	2
Disabled people				
Male patients	6-39	18·6	14	1
Patients with Duchenne muscular dystrophy				
Boys	6-22	17·2	31	31
Mothers:				
Obligatory carriers	39-57		21	12
Isolated cases	23-45	36·0	11	3
Sisters	11-25	17·1	10	5
Patients with other neuromuscular diseases				
Limb-girdle muscular dystrophy			2	2
Werdnig Hoffman paralysis	14-21	18	3	2
Spinal muscular atrophy	11-19		2	2
Peroneal muscular atrophy*			5	3-4
Dystrophia myotonica	15-62	39·0	12	9
Myasthenia gravis	16-61	43·8	10	0
Multiple sclerosis	29-64	46·7	9	0

*Including Charcot-Marie-Tooth atrophy.

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Haemolytic anaemia after cisplatin treatment

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Abstract

Normochromic or normocytic anaemia is a common side effect of treatment with cisplatin. Two patients treated with cisplatin 100 mg/m² in combination with vinblastine, bleomycin, and actinomycin D developed haemolytic anaemia. Neither patient had evidence of haemolysis before treatment, and in both cases severe haemolytic anaemia developed after several courses of cisplatin and when the cancer had regressed almost completely.

The importance of haemolysis in the development of anaemia after cisplatin treatment has not been investigated fully and further studies are needed.

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Introduction

cis-Dichlorodiammineplatinum (II) (cisplatin) is a cancer chemotherapeutic agent of value in several types of tumour.[1] Major side effects reported with cisplatin include gastrointestinal and renal toxicities, and ototoxicity[2]; anaemia has been reported in 9-40% of patients treated with cisplatin.[2] The anaemia is usually normochromic and normocytic in type and considered to be secondary to depression of erythropoiesis, though adequate studies to clarify the mechanisms responsible have not been reported. We report two cases of haemolytic anaemia occurring after cisplatin treatment.

Case reports

Case 1—A 37-year-old man with metastatic mixed embryonal cell carcinoma and teratocarcinoma of the testis affecting lungs and retroperitoneal lymph nodes started chemotherapy with cisplatin 100 mg/m² every three weeks in combination with vinblastine, bleomycin, and actinomycin D on 7 September 1978. His initial haemoglobin concentration was 16·3 g/dl. There was virtually complete regression of all metastatic disease over the next nine weeks. After the third course of cisplatin (total dose 503 mg) his haemoglobin

concentration was 8·7 g/dl, and four units of red cells were transfused. A fourth and last course of cisplatin (162 mg) was given a week later without apparent adverse effects. Ten days later his haemoglobin concentration had fallen to 8·0 g/dl, and he required further transfusion. His haemoglobin concentration fell rapidly to 7·2 g/dl over the next 10 days. Investigations showed a reticulocyte count of 33%, marked spherocytosis, five nucleated red cells per 100 white cells, serum lactic dehydrogenase activity of 630 U/l, and absent haptoglobins. The result of Schumms test was positive, as was a direct antiglobulin test, which showed specific reaction with C3 and C3e. Over the next two weeks he was given prednisone 60 mg/day and transfusion of R_2 R_2 red cells; his haemoglobin concentration rose to 13·8 g/dl, and all haematological and biochemical abnormalities resolved. Six months later he suffered a relapse and was given cisplatin in combination with doxorubicin and etoposide. Over the next two weeks his haemoglobin concentration fell from 11·5 g/dl to 8·5 g/dl. Despite transfusion, after a further course of cisplatin his haemoglobin concentration fell to 7·1 g/dl and the previously abnormal haematological and biochemical values returned. These recovered when cisplatin was withdrawn and a two-week course of prednisone was given. Partial regression of metastases was obtained with other agents, but five months later his disease became refractory and a further course of cisplatin was given with steroid cover. Despite this his haemoglobin concentration fell to 7·2 g/dl and no further cisplatin was given. The patient died six weeks later.

Case 2—A 33-year-old man with metastatic mixed embryonal cell carcinoma and teratocarcinoma of the testis affecting liver, lungs, and retroperitoneal lymph nodes started chemotherapy with cisplatin 100 mg/m^2 every three weeks in combination with vinblastine, bleomycin, and actionomycin D on 25 October 1977. His initial haemoglobin concentration was 11·3 g/dl. Major regression of all metastatic sites occurred over the next few weeks. On 8 April 1978, four days after his fifth and final course of cisplatin (total dose 900 mg), his haemoglobin concentration had fallen rapidly from 10·5 to 8·8 g/dl. A mild fever and rash developed during a subsequent red-cell transfusion. One month later his haemoglobin concentration was 8·0 g/dl, and a further febrile reaction occurred with transfusion. Within two weeks his haemoglobin concentration had fallen to 7·2 g/dl and he had associated fever and abdominal pain. Investigations showed a reticulocyte count of 11·4%, two nucleated red cells per 100 white cells, serum bilirubin concentration of 74 μmol/l (4·3 mg/100 ml), and absent haptoglobins. A Schumms test gave a positive result, direct antiglobulin test gave a negative result, and multispecific leucocyte antibodies were detected. Over the next six weeks despite high-dose prednisone and multiple transfusions of triple-washed red cells the haemolytic process continued. In a further attempt at control methotrexate 54 mg was given intravenously. Seven days later haemoglobin concentration was again 7·0 g/dl with associated neutropenia. Septicaemia developed, resulting in his death.

Discussions

Neither patient had evidence of haemolysis before cisplatin treatment, and in both cases a severe haemolytic anaemia developed after several courses of cisplatin and when the cancer had almost completely regressed. Only two previous cases of haemolysis with cisplatin have been reported, and it was suggested that an antibody reacting with a cisplatin-red-cell-membrane complex was responsible.[3] The first of our patients seems to have shown a similar mechanism, with haemolysis occurring on repeated exposure to the drug and the development of a positive direct antiglobulin test specific for C3 and C3e. The continued haemolysis for several weeks after exposure to the cisplatin could be explained by the long tissue retention of this agent.[4] Mechanisms for the haemolysis in the second patient are more difficult to categorise and may represent a transfusion reaction unrelated to cisplatin. Changes in red-cell membrane stability with bound cisplatin could also be postulated.

These cases raise the question of the relative importance of haemolysis in the development of anaemia after cisplatin treatment. Rothman and Weick showed that preferential reduction of erythroid stem cells was the principle mechanism for this anaemia in the single patient they studied,[5] which supported their unpublished data on in-vitro bone-marrow cultures after incubation with cisplatin. Haemolysis may well represent the less common mechanism, but further studies are needed to clarify the frequency and nature of the various factors concerned in cisplatin-induced anaemia.

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ONE HUNDRED YEARS AGO The following letter has been addressed by Mr Charles Darwin to Professor Holmgren, of Upsala, in answer to a request for an expression of his opinion on the question of the right to make experiments on living animals for scientific purposes—a question which is now being much discussed in Sweden.

"Down, Beckenham, April 14th, 1881.

"Dear Sir,—In answer to your courteous letter of April 7th, I have no objection to express my opinion with respect to the right of experimenting on living animals. I use this latter expression as more correct and comprehensive than that of vivisection. You are at liberty to make any use of this letter which you may think fit, but if published I should wish the whole to appear. I have all my life been a strong advocate for humanity to animals, and have done what I could in my writing to enforce this duty. Several years ago, when the agitation against physiologists commenced in England, it was asserted that inhumanity was here practised and useless suffering caused to animals; and I was led to think that it might be advisable to have an Act of Parliament on the subject. I then took an active part in trying to get a Bill passed, such as would have removed all just cause of complaint, and at the same time have left physiologists free to pursue their researches—a Bill very different from the Act which has since been passed. It is right to add that the investigation of the matter by a Royal Commission proved that the accusations made against our English physiologists were false. From all that I have heard, however, I fear that in some parts of Europe little regard is paid to the sufferings of animals, and if this be the case I should be glad to hear of legislation against inhumanity in any such country. On the other hand, I know that physiology cannot possibly progress except by means of experiments on living animals, and I feel the deepest conviction that he who retards the progress of physiology commits a crime against mankind. Any one who remembers, as I can, the state of this science half a century ago must admit that it has made immense progress and it is now progressing at an ever-increasing rate.

"What improvements in medical practice may be directly attributed to physiological research is a question which can be properly discussed only by those physiologists and medical practitioners who have studied the history of their subjects; but, as far as I can learn, the benefits are already great. However this may be, no one, unless he is grossly ignorant of what science has done for mankind, can entertain any doubt of the incalculable benefits which will hereafter be derived from physiology, not only by man, but by the lower animals. Look, for instance, at Pasteur's results in modifying the germs of the most malignant diseases from which, as it so happens, animals will in the first place receive more relief than man. Let it be remembered how many lives and what a fearful amount of suffering have been saved by the knowledge gained of parasitic worms through the experiments of Virchow and others on living animals. In the future, everyone will be astonished at the ingratitude shown, at least in England, to these benefactors of mankind. As for myself, permit me to assure you that I honour, and shall always honour, every one who advances the noble science of physiology.—Dear sir, yours faithfully, CHARLES DARWIN. —To Professor Holmgren." (*British Medical Journal*, 1881.)